Amendments to the Specification

Please replace the paragraph at column 2, line 65 to column 3, line 30 with the following replacement paragraph:

Human protein C is a serine protease zymogen present in blood plasma and synthesized in the liver. For expression of complete biological activity, protein C requires a post-translational modification for which vitamin K is needed. The mature, two-chain, disulfide-linked, protein C zymogen arises from a single-chain precursor by limited proteolysis. This limited proteolysis is believed to include cleavage of a signal peptide of ~33 amino acid residues (residues 1-33, below) during secretion of the nascent polypeptide from the liver, removal of a pro peptide of ~9 amino acid residues (residues 34-42), and removal of amino acid ~~resdues~~ residues 198 and 199 to form the two chains observed in the zymogen. The activation of the zymogen into the active serine protease involves the proteolytic cleavage of an ARG-LEU peptide bond (residues 211 and 212). This latter cleavage releases a dodecapeptide (residues 200-211) constituting the amino-terminus of the larger chain of the two-chain molecule. Protein C is significantly glycosylated; the mature enzyme contains ~23% carbohydrate. Protein C also contains a number of unusual amino acids, including γ-carboxyglutamic acid and β-hydroxyaspartic acid. γ-carboxyglutamic acid (gla) is produced from glutamic acid residues with the aid of a hepatic microsomal carboxylase which requires vitamin K as a cofactor. Since prokaryotes usually neither glycosylate, γ-carboxylate, nor β-hydroxylate proteins expressed from recombinant genes, the present invention is significant in that it allows for the first time the synthesis of protein C derivatives which have not undergone many of the post-translational modifications of normal human protein C. These unique derivatives have enormous research and clinical value, as discussed more fully below.

Please replace the paragraph at column 4, lines 28-30 with the following replacement paragraph:

Sensitive Host Cell--a host cell that cannot grow in the presence of a given antibiotic or other toxic compound without a DNA segment that confers resistance thereto.

Please replace the sequence paragraph at the bottom of columns 5 and 6 toward the end of columns 7 and 8 with the following replacement paragraph:

```
      [[5]]                5                  10                  15
H2N-MET TRP GLN LEU THR SER LEU LEU LEU PHE VAL ALA THR TRP GLY ILE

                 20                  25                  30
SER GLY THR PRO ALA PRO LEU ASP SER VAL PHE SER SER SER GLU ARG

          35                  40                  45
ALA HIS GLN VAL LEU ARG ILE ARG LYS ARG ALA ASN SER PHE LEU GLU

     50                  55                  60
GLU LEU ARG HIS SER SER LEU GLU ARG GLU CYS ILE GLU GLU ILE CYS
```

Serial No. 09/185,663

```
65                  70                  75                  80
ASP PHE GLU GLU ALA LYS GLU ILE PHE GLN ASN VAL ASP ASP THR LEU

                85                  90                  95
ALA PHE TRP SER LYS HIS VAL ASP GLY ASP GLN CYS LEU VAL LEU PRO

           100                 105                 110
LEU GLU HIS PRO CYS ALA SER LEU CYS CYS GLY HIS GLY THR CYS ILE

       115                 120                 125
ASP GLY ILE GLY SER PHE SER CYS ASP CYS ARG SER GLY TRP GLU GLY

   130                 135                 140
ARG PHE CYS GLN ARG GLU VAL SER PHE LEU ASN CYS SER LEU ASP ASN

145                 150                 155                 160
GLY GLY CYS THR HIS TYR CYS LEU GLU GLU VAL GLY TRP ARG ARG CYS

                165                 [[185]] 170         [[190]] 175
SER CYS ALA PRO GLY TYR LYS LEU GLY ASP ASP LEU LEU GLN CYS HIS

           180                 185                 190
PRO ALA VAL LYS PHE PRO CYS GLY ARG PRO TRP LYS ARG MET GLU LYS

       195                 200                 205
LYS ARG SER HIS LEU LYS ARG ASP THR GLU ASP GLN GLU ASP GLN VAL

   210                 215                 220
ASP PRO ARG LEU ILE ASP GLY LYS MET THR ARG ARG GLY ASP SER PRO

225                 230                 235                 240
TRP GLN VAL VAL LEU LEU ASP SER LYS LYS LYS LEU ALA CYS GLY ALA

                245                 250                 255
VAL LEU ILE HIS PRO SER TRP VAL LEU THR ALA ALA HIS CYS MET ASP

           260                 265                 270
GLU SER LYS LYS LEU LEU VAL SRG LEU GLY GLU TYR ASP LEU ARG ARG

       275                 280                 285
TRP GLU LYS TRP GLU LEU ASP LEU ASP ILE LYS GLU VAL PHE VAL HIS

   290                 295                 300
PRO ASN TYR SER LYS SER THR THR ASP ASN ASP ILE ALA LEU LEU HIS

305                 310                 315                 320
LEU ALA GLN PRO ALA THR LEU SER GLN THR ILE VAL PRO ILE CYS LEU

                325                 330                 335
PRO ASP SER GLY LEU ALA GLU ARG GLU LEU ASN GLN ALA GLY GLN GLU

           340                 345                 350
THR LEU VAL THR GLY TRP GLY TYR HIS SER SER ARG GLU LYS GLU ALA

       355                 360                 365
LYS ARG ASN ARG THR PHE VAL LEU ASN PHE ILE LYS ILE PRO VAL VAL

   370                 375                 380
PRO HIS ASN GLU CYS SER GLU VAL MET SER ASN MET VAL SER GLU ASN

385                 390                 395                 400
MET LEU CYS ALA GLY ILE LEU GLY ASP ARG GLN ASP ALA CYS CLU GLY

                405                 410                 415
ASP SER GLY GLY PRO MET VAL ALA SER PHE HIS GLY THR TRP PHE LEU

           420                 425                 430
VAL GLY LEU VAL SER TRP GLY GLU GLY CYS GLY LEU LEU HIS ASN TYR
```

```
                435                 440                 445
GLY VAL TYR THR LYS VAL SER ARG TYR LEU ASP TRP ILE HIS GLY HIS

    450                 455                 460

ILE ARG ASP LYS GLU ALA PRO GLN LYS SER TRP ALA PRO--COOH
```

Please replace the paragraph at column 8, line 66 to column 9, line 14 with the following replacement paragraph:

The DNA compounds of the present invention are derived from cDNA clones prepared from human liver mRNA that encodes human protein C activity. In constructing the cDNA clones, a 5' poly G sequence, a 3' poly C sequence, and both 5' and 3' PstI restriction enzyme recognition sequences were constructed at the ends of the protein C-encoding cDNA. Two of these cDNA clones were manipulated to construct a DNA molecule comprising both the coding sequence of nascent human protein C and also portions of the DNA encoding the untranslated mRNA at the 5' and [[3+]] 3' ends of the coding region. This DNA molecule was inserted into the PstI site of plasmid pBR322 to construct plasmid pHC7. Plasmid pHC7 thus comprises both the coding sequence above, wherein M and N both equal 1, and, again depicting only one strand of the molecule, also contains these additional sequences:

Please replace the paragraph at column 13, lines 2-14 with the following replacement paragraph:

Expression of the nascent human protein C structural gene contained on the above-described vectors occurs in those host cells in which the particular promoter associated with the nascent human protein C structural gene functions. The SV40 early promoter, the Rous Sarcoma virus long terminal repeat promoter, the Murine Sarcoma virus long terminal repeat promoter, and the mouse metallothionein promoter function in a wide variety of host cells. Preferred host cells for plasmids pSV2-HPC8, pL133, pL132, pL151, pL141, ~~pMSV-PC~~ pMSV-HPC, pMMTΔBPV-HPC and pL142 are listed in Table I, along with appropriate comments.

Please replace the paragraph at column 13, lines 42-58 with the following replacement paragraph:

Preferred transformants of the present invention are: HepG-2/pL132, HepG-2/pMSV-HPC, HepG-2/pL141 , HepG-2/pL151, HepG-2/pMMTΔBPV-HPC, H4IIEC3/pL141, H4IIEC3/pL132, H4IIEC3/pMMTΔBPV-HPC, H4IIEC3/pMSV-HPC, H4IIEC3/pL151, LLC-MK_2 /pL132, LLC-MK_2 /pMMTΔBPV-HPC, LLC-MK_2 /pL141, LLC-MK_2 /pL151, C127I/pMMTΔBPV-HPC, C127/pMSV-HPC, ~~C127I/pL151~~ 3T3/pMSV-HPC, 3T3/pMMTΔBPV-HPC, 3T3T3/pL132, 3T3/pL141, 3T3/pL151, RPMI8226/pMSV-HPC, RPMI8226/pMMTΔBPV-HPC, RPMI8226/pL132, RPMI8226/pL141, RPMI8226/pL151,

CHO-K1/pMSV-HPC, CHO-K1/pMMTΔBPV-HPC, CHO-K1/pL132, CHO-K1/pL141, CHO-K1/pL151, CHO-K1(dhfr⁻)/pMSV-HPC, ~~CHO-K1(dhfr⁻)/pMMTΔBPV-HPC~~ CHO-K1(dhfr⁻)/pL132, CHO-KI(dhfr⁻)/pL141, CHO-K1(dhfr⁻)/pL151.

Please replace the paragraph at column 14, line 60 to column 15, line 11 with the following replacement paragraph:

Before expressing the protein C activity-encoding DNA compounds of the present invention in prokaryotic host cells, the eukaryotic signal peptide-encoding DNA was removed. Theoretically, the first 33 amino acid residues at the amino-terminus of nascent human protein C act as a signal peptide to direct secretion of protein C from the liver into the bloodstream. The present invention is not limited to the use of a particular eukaryotic signal peptide for expression of protein C activity in eukaryotic host cells. As a general rule, prokaryotes do not efficiently process eukaryotic signal peptides; therefore, it would probably be somewhat inefficient to express the signal peptide-encoding portion of the nascent human protein C structural gene in prokaryotes. Although not ~~specificallly~~ specifically exemplified herein, the present invention also comprises the fusion of a prokaryotic signal peptide-encoding DNA to the protein C activity-encoding DNA of ~~teh~~ the present invention for expression and secretion of protein C activity in porkaryotes.

Please replace the paragraph at column 16, lines 23-41 with the following replacement paragraph:

The plasmid pNM789B starting material is derived from plasmid pKEN111 in accordance with the steps illustrated and described in FIGS. 10-17 and Example 10 below. Plasmid pKEN111 can be obtained from *E. coli* K12 CC620/pKEN111, a strain deposited and made part of the permanent stock culture collection of the Northern Regional Research Laboratory under the accession number NRRL B-15011. Plasmid pNM789B also contains the promoter and translational activating sequence of the *E. coli* lipoprotein gene and, in addition, the coding sequence, including an appropriately positioned translational stop signal, for a fusion protein comprising bovine growth hormone (bGH) and a nine amino acid residue polypeptide at the bGH amino-terminus. Ligation of the fusion protein-coding sequence, contained in the XbaI-BamHI fragment, to appropriately cleaved plasmid pIM-I'-A3 results in the aforementioned plasmid pCZ101 starting material.

Please replace the paragraph at column 18, lines 16-42 with the following replacement paragraph:

Many modifications and variations of the present illustrative DNA sequences and plasmids are possible. For example, the degeneracy of the genetic code allows for the substitution of nucleotides throughout polypeptide coding regions as well as for the

substitution of the

~~TAA~~ TAA or TGA
~~+++~~ ||| |||
~~ACT~~ ATT or ACT

translational stop signals for the

TAG
|||
ACT

translational for stop signal specifically exemplified. Such sequences can be deduced from the now-known amino acid or DNA sequence of human protein C and can be constructed by following conventional synthetic procedures. Such synthetic methods can be carried out in substantial accordance with the procedures of Itakura et al., 1977, Science 198:1056 and Crea et al., 1978, Proc. Nat. Acad. Sci. USA 75:5765. Therefore, the present invention is in no way limited to the DNA sequences and plasmids specifically exemplified.

Please replace the paragraph at column 22, line 50 to column 23, line 2 with the following replacement paragraph:

The culture prepared in Example 1A was centrifuged in a Sorvall GSA rotor (DuPont Co., Instrument Products, Biomedical Division, Newtown, CN 06470) at 6000 rpm for 5 minutes at 4° C. The resulting supernatant was discarded, and the cell pellet was washed in [[~0]] 40 ml of TES buffer (10 mM Tris-HCl, pH=7.5; 10 mM NaCl; and 1 mM EDTA) and then repelleted. After discarding the supernatant again, the cell pellet was frozen in a dry ice-ethanol bath and then thawed. The thawed cell pellet was resuspended in 10 ml of a 25% sucrose/50 mM EDTA solution. After adding and mixing: 1 ml of a 5 mg/ml lysozyme solution; 3 ml of 0.25M EDTA, pH=8.0; and 100 µl of 10 mg/ml RNAse A, the solution was incubated on ice for 15 minutes. Three ml of lysing solution (prepared by mixing 3 ml 10% Triton-X 100; 75 ml 0.25M EDTA, pH=8.0; 15 ml of 1M Tris-HCl, pH=8.0; and 7 ml of water) were added to the lysozyme-treated cells, mixed, and the resulting solution incubated on ice for another 15 minutes. The lysed cells were frozen in a dry ice-ethanol bath and then thawed.

Please replace the paragraph at column 27, lines 29-30 with the following replacement paragraph:

B. Isolation of the ~1.15kb SalI-BglII Restriction Fragment of Plasmid pSV2-HPC8.

Please replace the paragraph at column 29, lines 8-25 with the following replacement paragraph:

XhoI linkers (New England Biolabs, 32 Tozer Road, Beverly, MA 09195) of sequence:

~~5'-CCTCGAGG-3'~~ 5'-CCTCGAGG-3'
~~3'-GGAGCTCC-5'~~ 3'-GGAGCTCC-5'

were kinased and prepared for ligation by the following procedure. Four µl of linkers (~2 µg) were dissolved in 20.15 µl of H_2O and 5 µl of 10X kinase buffer (500 mM Tris-HCl, pH=7.6 and 100 mM $MgCl_2$), incubated at 90° C. for two minutes, and then cooled to room temperature. Five µl of γ-^{32}P-ATP (~20 µCi), 2.5 µl of 1M DTT, and 5 µl of polynucleotide kinase (~10 units) were added to the mixture, which was then incubated at 37.degree. C. for 30 minutes. Then, 3.35 µl of 0.01 mM ATP and 5 more µl of kinase were added, and the reaction was continued for another 30 minutes at 37° C. The reaction was then stored at –20° C.

Please replace the paragraph at column 31, lines 17-29 with the following replacement paragraph:

Fifty µg of plasmid pRSVcat (available from the ATCC in host *E. coli* ~~HB110~~ HB101 under accession number ATCC 37152) were mixed with 10 µl 10X HindIII reaction buffer, 5 µl (~50 units) restriction enzyme HindIII, and 85 µl of H_2O, and the resulting digest was incubated at 37° C. for 2 hours. After the HindIII digestion, the DNA was precipitated and collected by centrifugation. The DNA pellet was dissolved in 10 µl 10X NdeI reaction buffer (1.5M NaCl; 100 mM Tris-HCl, pH=7.8; 70 mM $MgCl_2$; and 60 mM 2-mercaptoethanol), 10 µl (~30 units) restriction enzyme NdeI, and 85 µl of H_2O, and the resulting reaction was incubated at 37° C. until the digestion was complete.

Please replace the paragraph at column 32, lines 46-59 with the following replacement paragraph:

After a five minute incubation at 65° C., the BamHI-digested plasmid pdBPV-MMTneo DNA was diluted to a concentration of about 0.1 µg/µl in ligase buffer and ligated with T4 DNA ligase, and the resulting plasmid ~~pMMTΔABPV~~ pMMTΔBPV DNA was used to transform *E. coli* K12 RR1 in substantial accordance with the teaching of Examples 2H and 2I. The *E. coli* K12 RR1/pMMTΔBPV transformants were identified by their ampicillin-resistant phenotype and by restriction enzyme analysis of their plasmid DNA. Plasmid pMMTΔBPV DNA was isolated from the transformants in substantial accordance with the teaching of Example 1, except that ampicillin was the antibiotic used during culturing of the cells.

Please replace the paragraph at column 43, lines 20-40 with the following replacement paragraph:

The bacterium *E. coli* K12/pIM-Γ-A3 (NRRL B-15733) was cultured in TY broth (1%

tryptone, 0.5% yeast extract, 0.5% sodium chloride, and pH 7.4) with 50 µg/ml of kanamycin at 25° C. according to conventional microbiological procedures. After the culture was diluted 1:10 into fresh broth and after 3 hours incubation at 37° C., about 1.5 ml of the culture were transferred to a 1.5 ml Eppendorf tube and centrifuged for about 15 seconds. Unless otherwise indicated, all the manipulations were done at ambient temperature. The resultant supernatant was carefully removed with a fine-tip ~~aspiratr~~ aspirator, and the cell pellet was resuspended in about 100 µl of freshly prepared lysozyme solution which contained 2 mg/ml lysozyme, 50 mM glucose, 10 mM EDTA, and 25 mM Tris.HCl at pH 8. About 200 µl of alkaline SDS (sodium dodecyl sulfate) solution (0.2N NaOH and 1% SDS) were added and the tube was gently inverted and then kept at 0° C. until lysis was complete (~5 minutes). Next, about 150 µl of 3M sodium acetate were added, and the contents of the tube mixed gently by inversion.

Please replace the paragraph at column 45, lines 61-68 with the following replacement paragraph:

DNA sequencing of plasmid pCZ141 revealed that the BamHI overlaps were not "filled-in" as expected ~~whn~~ when treating with Klenow enzyme. Instead, the BamHI overlaps and some adjoining sequences were removed from the plasmid pCZ118 DNA before the NdeI linkers were attached. This contaminating nuclease activity did not affect, in any material way, the subsequent steps in the construction of plasmid pCZ459.